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“Evaluating In Vivo Efficacy of Enzymatic Biofilm Dispersal from Orthopaedic Implants”

INTRODUCTION: Biofilm related implant complications affect 0.5-2% of all orthopaedic procedures, highlighting the urgent need for effective cleaning methods. Recent in vitro studies have shown that bromelain enzymatic debridement can effectively break down biofilms on infected implants, but its efficacy in vivo has yet to be investigated. Through utilization of the nucleic acid stain Sytox Orange, we can assess the bioburden on both infected and uninfected explanted in vivo orthopaedic implants. By fluorescently labeling sessile bacteria in biofilm, this stain allows for a comparison of bacterial burden in vitro as well as in vivo. This study aims to gain insights into bromelain's capability to remove biofilms outside of culture through an in vivo model.

METHODS: Stainless steel bone pins were incubated at 37°C in tryptic soy broth with 10% fetal bovine serum and inoculated with methicillin-resistant *Staphylococcus aureus* (MRSA) for 120 hours inside a 12-well plate. Biofilm washed pins were first infected in the same fashion and then soaked in 1000 µg/mL bromelain for 20 minutes followed by phosphate buffered saline (PBS) rinse. Uninfected, infected, or infected and bromelain washed pins were placed in zinc-buffered formalin fixative to demonstrate the in vitro condition of the biofilm. A second set of pins prepared as above were placed into the intramedullary space of adult Sprague-Dawley rats for seven days. After seven days, the pins were explanted and placed in fixative. All pins were washed with PBS after fixation. The pins were stained with Sytox Orange (Thermo) for 10 minutes and then washed with PBS. Subsequently, the pins were mounted on slide spacers with antifade media and mounted on a slide. The pin threads were imaged at 100x magnification using confocal microscopy. The bacterial burden was quantified by size exclusion relative to host cells for explanted pins using SlideBook 5.0 software (3i). Statistical comparison between pins exposed to in vitro or in vivo conditions was done using Prism 10.0 (GraphPad) with one-way ANOVA and $\alpha = 0.05$.

RESULTS: The in vitro infected pins (n=6) exhibited a mean bacterial burden of 560.83 colony forming units (CFU), while the in vivo infected pins (n=3) displayed a higher mean burden of 873.7 CFU (p=0.3944). The pre-washed pins (n=3) had a mean bacterial burden of 819.3 CFU compared to the in vitro bromelain-washed pins (n=4), demonstrating a vastly lower bacterial burden of 96.5 CFU (p=0.0195).

DISCUSSION: The data indicate that while bromelain shows promise as a viable option for cleaning infected orthopedic implants, further exploration beyond in vitro culture studies is necessary. Although the bromelain washes effectively reduced the bacterial burden in vitro, it was insufficient in maintaining low bacterial levels in vivo. Additionally, a larger sample size is required to validate the credibility of our findings. Stricter sterile techniques must be implemented in future research to prevent extraneous contamination.